

**Data Evaluation Record on the Acute Toxicity of Formulated Flufenacet (Axiom 68 DF, 54.4% a.i. Flufenacet, 13.8% a.i. Metribuzin) to Terrestrial Vascular Plants: Seedling Emergence and Vegetative Vigor**

**PMRA Submission Number {.....}**

**EPA MRID Number 48897608**

<b>Data Requirement:</b>	PMRA Data Code:	9.8.4 (TGAI) or 9.8.6 (EP)
	EPA DP Barcode:	405834
	OECD Data Point:	IIA 8.12 (TGAI) and IIIA 10.8.1.1 (EP)
	EPA Guideline:	850.4100 and 850.4150

**Test material: Axiom 68 DF formulation**

A.I. Flufenacet

**Purity:** 54.4%

A.I. Metribuzin

**Purity:** 13.8%

Common name: Axiom 68 DF

Chemical name:

IUPAC (Flufenacet): N-(4-Fluorophenyl)-N-(1-methylethyl)-2-[[5-(trifluoromethyl)-1,3,4-thiadiazol-2-yl]oxy]acetamide

IUPAC (Metribuzin): 4-Amino-6-(1,1 -dimethylethyl)-3-(methylthio)-1,2,4-triazin-5(4H)-one

CAS name: Not reported

CAS No. (Axiom 68 DF): 153409-61-1

Synonyms: Axiom 68 DF

**Primary Reviewer:** Joan Gaidos  
**Senior Scientist, CDM Smith**

**Signature:**

**Date:** 7/15/15

**Secondary Reviewer:** Teri S. Myers  
**Senior Scientist, CDM Smith**

**Signature:**

**Date:** 7/31/15

**Primary Reviewer:** Geoffrey Sinclair  
**EPA/OPP/EFED/ERB5**

**Date:** 11/17/15

**Secondary Reviewer(s):** {.....}  
**{EPA/OECD/PMRA}**

**Date:** {.....}

**Reference/Submission No.:** {.....}

**Company Code** {.....} [For PMRA]

**Active Code** {.....} [For PMRA]

**Use Site Category:** {.....} [For PMRA]

**EPA PC Code** 121903 (Flufenacet)

101101 (Metribuzin)

**Date Evaluation Completed:** {dd-mm-yyyy}

**CITATION:** Tier 2 Seedling Emergence and Vegetative Vigor Nontarget Phytotoxicity Study Using Axiom 68 DF. Unpublished study performed and sponsored by Bayer Corporation, Agricultural Division, Stilwell, Kansas and Kansas City, Missouri. Laboratory Project Number: 109592. Study completed June 12, 2000.

**DISCLAIMER:** This document provides guidance for EPA and PMRA reviewers on how to complete a data evaluation record after reviewing a scientific study concerning the acute toxicity of a pesticide to terrestrial vascular plants. It is not intended to prescribe conditions to any external party for conducting this study nor to establish absolute criteria regarding the assessment of whether the study is scientifically sound and whether the study satisfies any applicable data requirements. Reviewers are expected to review and to determine for each study, on a case-by-case basis, whether it is scientifically sound and provides sufficient information to satisfy applicable data requirements. Studies that fail to meet any of the conditions may be accepted, if appropriate; similarly, studies that meet all of the conditions may be rejected, if appropriate. In sum, the reviewer is to take into account the totality of factors related to the test methodology and results in determining the acceptability of the study.

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## EXECUTIVE SUMMARY:

The effect of the formulation Axiom 68 DF (**54.4% a.i. Flufenacet; 13.8% a.i. Metribuzin**) on the **seedling emergence** of monocot (onion, *Allium cepa*; sorghum, *Sorghum bicolor*; wheat, *Triticum aestivum*) and dicot (turnip, *Brassica rapa*) crops and on the **vegetative vigor** of monocot (onion, *Allium cepa*; sorghum, *Sorghum vulgare*; wheat, *Triticum aestivum*) and dicot (turnip, *Brassica rapa*) crops were studied at a nominal concentration of 0 (negative), 0 (solvent), 0.004, 0.008, 0.016, 0.032, 0.064, 0.128 and 0.256 lb ai formulation/A with the a.i. representing 68.2% combined active ingredients, Flufenacet and Metribuzin for the formulation of Axiom 68 DF. The nominal test concentrations for Flufenacet were 0 (negative), 0 (solvent), 0.0032, 0.0064, 0.013, 0.026, 0.051, 0.103 and 0.21 lb ai/A Flufenacet, and 0 (negative), 0 (solvent), 0.00082, 0.0016, 0.0033, 0.0065, 0.013, 0.026 and 0.052 lb ai/A Metribuzin.

Treatment rates were analytically confirmed in the dosing solutions at each application rate for the individual active ingredients, Flufenacet and Metribuzin. Recoveries ranged from 44-68% of nominal for Flufenacet and 91-97% of nominal for Metribuzin. Method recoveries indicated similarly recovery trends for Flufenacet of 29-39% of nominal and Metribuzin 90-94% of nominal. The study author determined the Flufenacet analysis was invalid due to a filtration step that trapped Flufenacet particles, introducing error into the analytical measurement. Therefore, to obtain the nominal and measured Flufenacet only application rates, the reviewer back-calculated based on the measured Metribuzin concentrations.

Calculated measured Flufenacet and Metribuzin concentrations were 0.0030, 0.0059, 0.012, 0.024, 0.048, 0.097 and 0.19 lb ai/A Flufenacet, and 0.00075, 0.0015, 0.0032, 0.0060, 0.012, 0.024 and 0.048 lb ai/A Metribuzin.

The growth medium used in the seedling emergence and vegetative vigor tests was standard soil (sandy loam, pH 5.7, organic carbon not reported; organic matter 2.5%). On day 21 the surviving plants per pot were recorded and height and dry weight per replicate determined.

Both studies (seedling emergence and vegetative vigor) are classified as supplemental due to insufficient number of species tested.

### Seedling Emergence

Negative control emergence ranged from 80 to 100%. There were no significant effects on seedling emergence for any species tested.

The reviewer measured seedling survival based on number planted. Negative control survival based on number planted ranged from 78 to 100%. There were significant inhibitions in survival in all species except wheat. Significant inhibitions in turnip measured 32, 76 and 92% at the 0.024, 0.048 and 0.097 lb ai/A Flufenacet treatment levels, respectively, compared to the negative control and exhibited a dose-response (Williams test,  $p < 0.05$ ). Significant inhibitions in onion survival were 37 and 63% at the 0.048 and 0.097 lb ai/A Flufenacet treatment levels, respectively; sorghum significant inhibitions were observed at the highest treatment level only, 0.048 lb ai/A Flufenacet, and measured 48% (Williams test,  $p < 0.050$ ).

There were significant inhibitions in seedling height in all species compared to the negative control. Significant inhibitions in onion height were 10, 20, 35 and 40% at the 0.012, 0.024, 0.048 and 0.097 lb ai/A Flufenacet treatment levels, respectively, and significant inhibitions in sorghum height were 12, 22 and 37% at the 0.012, 0.024 and 0.048 lb ai/A Flufenacet treatment levels, respectively (Williams test,  $p < 0.05$ ). Significant inhibitions in turnip and wheat were observed at the highest treatment level only, 0.097 lb ai/A Flufenacet, and measured 50 and 10%, respectively, compared to the negative control (Williams test,  $p < 0.05$ ).

Significant inhibitions in seedling dry weight were observed in onion, sorghum and turnip, but not in wheat.

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Significant inhibitions in sorghum dry weight of 18, 31 and 40% were found at 0.012, 0.024 and 0.048 lb ai/A Flufenacet treatment levels, respectively, compared to the negative control, exhibiting a dose-response (Williams test,  $p < 0.05$ ). Onion significant inhibitions in seedling dry weight were 41 and 31% at the 0.048 and 0.097 lb ai/A Flufenacet treatment levels, and turnip significant inhibitions in dry weight were observed at the highest treatment level only, 0.097 lb ai/A Flufenacet, and measured 61% (Dunnett's test,  $p < 0.05$ ).

Based on the reviewer's seedling emergence results, the most sensitive monocot species was sorghum based on dry weight, with NOAEC and  $IC_{25}$  values of 0.0059 and 0.0175 lb ai/A Flufenacet, respectively; and the most sensitive dicot species was turnip based on survival, with NOAEC and  $EC_{25}$  values of 0.012 and 0.0225 lb ai/A Flufenacet, respectively. In terms of the other active ingredient, Metribuzin, NOAEC and  $IC_{25}$  values for sorghum dry weight were 0.0015 and 0.00444 lb ai/A Metribuzin, respectively, and the NOAEC and  $EC_{25}$  values for turnip survival were 0.0030 and 0.0057 lb ai/A Metribuzin, respectively.

Based on reviewer calculated total formulation concentrations, the NOAEC and  $IC_{25}$  values for sorghum based on dry weight were 0.011 and 0.0322 lb/A total formulation, respectively, and the NOAEC and  $EC_{25}$  values for turnip based on survival were 0.022 and 0.041 lb/A total formulation, respectively.

The occurrence of phytotoxic effects increased in severity and prevalence with increasing test concentrations for all species except wheat. Maximum effects were 75, 60 and 100% for onion, sorghum and turnip, respectively. Effects were dose-related.

### **Vegetative Vigor**

The reviewer could not validate the seedling survival, height or dry weight data for cucumber and turnip in this study because it appears that either dead plants were measured, or the survival data was in error. As a result, only the sorghum data was analyzed for trends. There were no significant inhibitions in seedling survival for sorghum compared to the negative control.

There were significant inhibitions in seedling height in sorghum compared to the negative control. Significant decreases in seedling height in sorghum were 11 and 34% at the 0.048 and 0.097 lb ai/A Flufenacet treatment levels, respectively (Williams test,  $p < 0.05$ ).

There were also significant inhibitions in seedling dry weight in sorghum compared to the negative control. Significant inhibitions in sorghum dry weight of 15, 23, 21 and 32% were observed at the 0.012, 0.024, 0.048 and 0.097 lb ai/A Flufenacet/A treatment levels, respectively (Williams test,  $p < 0.05$ ). There was also a significant difference between the negative control and the solvent control for sorghum dry weight (19%; t-test,  $p = 0.0298$ ).

Based on the reviewer's vegetative vigor results, the most sensitive endpoint for the monocot sorghum was dry weight, with NOAEC and  $IC_{25}$  values of 0.0059 and 0.0491 lb ai/A Flufenacet, respectively. The most sensitive dicot could not be determine due to invalid data. In terms of the other active ingredient, Metribuzin, NOAEC and  $IC_{25}$  values for sorghum dry weight were 0.0015 and 0.0125 lb ai/A Metribuzin, respectively.

Based on reviewer calculated total formulation concentrations, the NOAEC and  $IC_{25}$  values for sorghum based on dry weight were 0.011 and 0.090 lb/A total formulation, respectively.

The occurrence of phytotoxic effects increased in severity and prevalence with increasing test concentrations for sorghum. Maximum effects were 40% for sorghum. Effects were dose-related.

**Maximum Labeled Rate: Not reported**

### **Results Synopsis**

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**Seedling Emergence: Flufenacet**

**Monocot**

**Most sensitive monocot: Sorghum, based on dry weight**

EC <sub>50</sub> /IC <sub>50</sub> : 0.0549 lb ai/A Flufenacet	95% C.I.: 0.035-0.0862 lb ai/A Flufenacet
EC <sub>25</sub> /IC <sub>25</sub> : 0.0175 lb ai/A Flufenacet	95% C.I.: 0.0114-0.0251 lb ai/A Flufenacet
EC <sub>05</sub> /IC <sub>05</sub> : 0.00337 lb ai/A Flufenacet	95% C.I.: N/A-0.00768 lb ai/A Flufenacet
NOEC: 0.0059 lb ai/A Flufenacet	
Slope: N/A	95% C.I.: N/A

**Dicot**

**Most sensitive dicot: Turnip, based on survival**

EC <sub>50</sub> /IC <sub>50</sub> : 0.0335 lb ai/A Flufenacet	95% C.I.: 0.0288-0.0391 lb ai/A Flufenacet
EC <sub>25</sub> /IC <sub>25</sub> : 0.0225 lb ai/A Flufenacet	95% C.I.: 0.0184-0.0264 lb ai/A Flufenacet
EC <sub>05</sub> /IC <sub>05</sub> : 0.0127 lb ai/A Flufenacet	95% C.I.: 0.00904-0.0159 lb ai/A Flufenacet
NOEC: 0.012 lb ai/A Flufenacet	
Slope: 3.9	95% C.I.: 2.98-4.83

**Seedling Emergence: Metribuzin**

**Monocot**

**Most sensitive monocot: Sorghum, based on dry weight**

EC <sub>50</sub> /IC <sub>50</sub> : 0.014 lb ai/A Metribuzin	95% C.I.: 0.0089-0.022 lb ai/A Metribuzin
EC <sub>25</sub> /IC <sub>25</sub> : 0.00444 lb ai/A Metribuzin	95% C.I.: 0.0029-0.0064 lb ai/A Metribuzin
EC <sub>05</sub> /IC <sub>05</sub> : 0.00085 lb ai/A Metribuzin	95% C.I.: N/A-0.0019 lb ai/A Metribuzin
NOEC: 0.0015 lb ai/A Metribuzin	
Slope: N/A	95% C.I.: N/A

**Dicot**

**Most sensitive dicot: Turnip, based on survival**

EC <sub>50</sub> /IC <sub>50</sub> : 0.0085 lb ai/A Metribuzin	95% C.I.: 0.0073-0.010 lb ai/A Metribuzin
EC <sub>25</sub> /IC <sub>25</sub> : 0.0057 lb ai/A Metribuzin	95% C.I.: 0.0047-0.0067 lb ai/A Metribuzin
EC <sub>05</sub> /IC <sub>05</sub> : 0.0032 lb ai/A Metribuzin	95% C.I.: 0.0023-0.0040 lb ai/A Metribuzin
NOEC: 0.0030 lb ai/A Metribuzin	
Slope: 0.989	95% C.I.: 0.756-1.225

**Vegetative Vigor: Flufenacet**

**Monocot**

**Most sensitive monocot: Sorghum, based on dry weight**

EC <sub>50</sub> /IC <sub>50</sub> : 0.757 lb ai Flufenacet/A	95% C.I.: 0.081-7.07 lb ai Flufenacet/A
EC <sub>25</sub> /IC <sub>25</sub> : 0.0491 lb ai Flufenacet/A	95% C.I.: 0.0253-0.089 lb ai Flufenacet/A
EC <sub>05</sub> /IC <sub>05</sub> : 0.000958 lb ai Flufenacet/A	95% C.I.: N/A-0.0156 lb ai Flufenacet/A
NOEC: 0.0059 lb ai Flufenacet/A	
Slope: N/A	95% C.I.: N/A

**Dicot**

**Most sensitive dicot: Could not be determined, invalid data for dicots**

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EC<sub>50</sub>/IC<sub>50</sub>: N/A 95% C.I.: N/A  
 EC<sub>25</sub>/IC<sub>25</sub>: N/A 95% C.I.: N/A  
 EC<sub>05</sub>/IC<sub>05</sub>: N/A 95% C.I.: N/A  
 NOEC: N/A  
 Slope: N/A 95% C.I.: N/A

**Vegetative Vigor: Metribuzin**

**Monocot**

**Most sensitive monocot: Sorghum, based on dry weight**

EC<sub>50</sub>/IC<sub>50</sub>: 0.192 lb ai/A Metribuzin 95% C.I.: 0.021-1.793 lb ai/A Metribuzin  
 EC<sub>25</sub>/IC<sub>25</sub>: 0.0125 lb ai/A Metribuzin 95% C.I.: 0.0064-0.023 lb ai/A Metribuzin  
 EC<sub>05</sub>/IC<sub>05</sub>: 0.00024 lb ai/A Metribuzin 95% C.I.: N/A-0.0018 lb ai/A Metribuzin  
 NOEC: 0.0015 lb ai/A Metribuzin  
 Slope: N/A 95% C.I.: N/A

**Dicot**

**Most sensitive dicot: Could not be determined, invalid data for dicots**

EC<sub>50</sub>/IC<sub>50</sub>: N/A 95% C.I.: N/A  
 EC<sub>25</sub>/IC<sub>25</sub>: N/A 95% C.I.: N/A  
 EC<sub>05</sub>/IC<sub>05</sub>: N/A 95% C.I.: N/A  
 NOEC: N/A  
 Slope: N/A 95% C.I.: N/A

**Table 1a. Summary of most sensitive parameters by species (lb ai/A Flufenacet). Seedling Emergence**

Species	Endpoint	NOEC	EC <sub>05</sub> /IC <sub>05</sub>	EC <sub>25</sub> /IC <sub>25</sub>	EC <sub>50</sub> /IC <sub>50</sub>
Onion	Survival	0.024	0.00573	0.0218	0.0552
Sorghum	Dry Weight <sup>a</sup>	0.0059	0.00337	0.0175	0.0549
Turnip	Survival	0.012	0.0127	0.0225	0.0335
Wheat	None <sup>b</sup>	0.048	>0.097	>0.097	>0.097

ND- Not determined. NC- Not calculable.

<sup>a</sup> Sorghum survival had a lower IC<sub>25</sub>/EC<sub>25</sub> than sorghum dry weight, however sorghum survival EC<sub>25</sub> was less than the NOAEC and may have been influenced by the solvent and low seedling emergence and survival.

<sup>b</sup> There was a significant decrease in wheat height of 10% at the highest treatment level, 0.097 lb ai/A Flufenacet, however, the endpoints were higher than the tested concentrations.

**Table 1b. Summary of most sensitive parameters by species (lb ai/A Metribuzin). Seedling Emergence**

Species	Endpoint	NOEC	EC <sub>05</sub> /IC <sub>05</sub>	EC <sub>25</sub> /IC <sub>25</sub>	EC <sub>50</sub> /IC <sub>50</sub>
Onion	Survival	0.0060	0.0015	0.00553	0.014
Sorghum	Survival	0.0060	0.00034	0.000974	0.0099
Turnip	Survival	0.0030	0.0032	0.0057	0.0085
Wheat	None <sup>a</sup>	0.012	>0.024	>0.024	>0.024

ND- Not determined. NC- Not calculable.

<sup>a</sup> Sorghum survival had a lower IC<sub>25</sub>/EC<sub>25</sub> than sorghum dry weight, however sorghum survival EC<sub>25</sub> was less than the NOAEC and may have been influenced by the solvent and/or low seedling emergence and survival.

<sup>b</sup> There was a significant decrease in wheat height of 10% at the highest treatment level, 0.024 lb ai/A Metribuzin, however, the endpoints were higher than the tested range of concentrations.

**Table 1c. Summary of most sensitive parameters by species (lb ai/A Flufenacet). Vegetative Vigor**

Species	Endpoint	NOEC	EC <sub>05</sub> /IC <sub>05</sub>	EC <sub>25</sub> /IC <sub>25</sub>	EC <sub>50</sub> /IC <sub>50</sub>
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Cucumber	ND	ND	ND	ND	ND
Sorghum	Dry weight	0.0059	0.000958	0.0491	0.757*
Turnip	ND	ND	ND	ND	ND

ND- Not determined. NC- Not calculable.

\*Endpoint and/or confidence limits are outside the tested range of concentrations and should be interpreted cautiously. Survival, height and weight data for cucumber and turnip were invalidated.

**Table 1d. Summary of most sensitive parameters by species (lb ai/A Metribuzin). Vegetative Vigor**

Species	Endpoint	NOEC	EC <sub>05</sub> /IC <sub>05</sub>	EC <sub>25</sub> /IC <sub>25</sub>	EC <sub>50</sub> /IC <sub>50</sub>
Cucumber	ND	ND	ND	ND	ND
Sorghum	Dry weight	0.0015	0.00024	0.012	0.192*
Turnip	ND	ND	ND	ND	ND

ND- Not determined. NC- Not calculable.

\*Endpoint and/or confidence limits are outside the tested range of concentrations and should be interpreted cautiously. Survival, height and weight data for cucumber and turnip were invalidated.

1. This study is scientifically sound but is classified a supplemental (quantitative for only those species that have valid results) because it did not achieve any results for dicots in the vegetative vigor toxicity study and tested insufficient number of species. In the Vegetative Vigor Study, height and weight measurements were provided for treatment level data with up to 100% mortality, which means dead plants may have been measured and weighed and the data included in the report or there was an error the survival data. There was no way to correct the data.

## **I. MATERIALS AND METHODS**

### **GUIDELINE FOLLOWED:**

This study was conducted in compliance with FIFRA Good Laboratory Practice Standards as published by the U.S. EPA, 40 CFR Part 160 (1989). The reviewer evaluated the study methods according to EPA Ecological Effects Test Guidelines, OCSPP Guideline 850.4100: Seedling Emergence and Seedling Growth and OCSPP Guideline 850.4150: Vegetative Vigor. There were some deficiency and deviations noted by the reviewer.

1. Applying to both the Seedling Emergence and Vegetative Vigor study, analytical confirmation of Flufenacet test concentrations was invalid. Measured Flufenacet recoveries were low (44-68%) as were the method validation recoveries (29-39%). The study author determined the analysis was invalid due to a filtration step that trapped Flufenacet particles, introducing error into the analytical measurement. Therefore, to obtain the measured Flufenacet application rates, the reviewer back calculated Flufenacet concentrations from the measured Metribuzin concentrations (Metribuzin recoveries were valid, 91-97%; method validation recoveries 90-94%).
2. Applying to both the Seedling Emergence and Vegetative Vigor study, the study author reported that sorghum, wheat, onion and cucumber had previously been identified to be the most sensitive of the ten crops tested with Flufenacet (Johns, 1994). Turnip, onion and cucumber had previously been identified to be the most sensitive of 10 crops tested with Metribuzin (Bürge, 1992). This study utilized 3 monocots and 1 dicot for the seedling emergence study and 1 monocot and 2 dicots for the vegetative vigor study. EPA recommends four monocots in two families, including corn and six dicots in four families, including soybean and a root crop. This study cannot be upgraded or used for risk assessment purposes until the 5 remaining dicots and one remaining monocot are included in the analysis.
3. Applying to both the Seedling Emergence and Vegetative Vigor study, the study author presented data for

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seedling dry weight as a total weight and did not take into account number of seedlings per replicate.

4. Mean control seedling survival was 78% for sorghum in the Seedling Emergence study, which is lower than the OCSPP recommended mean seedling survival of 90%. Historic germination rates were not provided.
5. In the Seedling Emergence study, sorghum EC<sub>25</sub> for survival was much lower than the NOAEC and may be related to the solvent (24% different between the negative control survival and solvent control survival) and/or low seedling emergence and survival. The reviewer selected sorghum dry weight as the endpoint instead of sorghum survival because sorghum dry weight had a definitive dose-response.
6. In the Vegetative Vigor Study, height and weight measurements were provided for treatment level data with up to 100% mortality, which means dead plants may have been measured and weighed and the data included in the report or there was an error the survival data. There was no way to correct the data.
7. The physico-chemical properties of the test material were not reported. Soil % organic carbon, CEC and moisture content were not reported.

The deficiency and deviations did have an impact on the acceptability of this study causing the study to be classified as supplemental.

## COMPLIANCE:

Signed and dated GLP, Quality Assurance and Data Confidentiality statements were provided. This study was conducted in compliance with Good Laboratory Practice Standards as published by U.S. EPA, 40 CFR Part 160 (1989).

## A. MATERIALS:

### 1. Test Material

**Axiom 68 DF (54.4% a.i. Flufenacet; 13.8% a.i. Metribuzin)**

#### Description:

Tan granules.

#### Lot No./Batch No.:

903-0070

#### Purity:

99.3% (Flufenacet, 54.4%)  
90.8% (Metribuzin, 13.8%)

#### Stability of compound under test conditions:

Measured Flufenacet recoveries in dosing solutions were low, 44-68% (n = 7) as were the method validation recoveries, 29-39% (n = 5). The study author determined the analysis was invalid due to a filtration step that trapped Flufenacet particles, introducing error in to the analytical measurement. Measured Metribuzin recoveries in the dosing solutions were 91-97% (n=7) and method validation recoveries 90-94% (n = 5). Stability was not determined.

*(OECD recommends chemical stability in water and light)*

#### Storage conditions of test chemicals:

The test material was stored frozen.



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**Table 2. Physical/chemical properties of Flufenacet**

Parameter	Values	Comments
Water solubility at 20°C	Not reported	
Vapor pressure	Not reported	
UV absorption	Not reported	
pKa	Not reported	
Kow	Not reported	

**2. Test organism:**

**Monocotyledonous species:** Onion (*Allium cepa*, Amaryllidaceae, Southport), sorghum (*Sorghum bicolor*, Gramineae, VP53), and wheat (*Triticum aestivum*, Gramineae, Common); *EPA recommends four monocots in two families, including corn.*

**Dicotyledonous species:** Cucumber (*Cucumis sativus*; Cucurbitaceae, Straight Eight); and Turnip (*Brassica rapa*, Brassicaceae, Purple Top); *EPA recommends six dicots in four families, including soybean and a root crop.*

*OECD recommends a minimum of three species selected for testing, at least one from each of the following categories: Category 1: ryegrass, rice, oat, wheat, and sorghum; Category 2: mustard, rape, radish, turnip, and Chinese cabbage; Category 3: vetch, mung bean, red clover, fenugreek, lettuce, and cress.*

**Seed source:** All seeds supplied by Planters with the exception of sorghum, supplied by Valley Seed.

**Prior seed treatment/sterilization:** The seeds were not treated with any type of fungicides, insecticides, or any pesticides.

**Historical % germination of seed:** Historic germination rates were not reported.

**Seed storage, if any:** Not reported.

**B. STUDY DESIGN:**

**1. Experimental Conditions**

- a. Limit test: None.
- b. Range-finding study: None.
- c. Definitive Study

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**Table 3: Experimental Parameters - Seedling Emergence/Vegetative Vigor.**

Parameters	Seedling Emergence/Vegetative Vigor	
	Details	Remarks
		Criteria
Duration of the test	21 days	<p><i>Recommended test duration is 14-21 days.</i></p> <p><i>Seedling Emergence: OECD recommends that the test be terminated no sooner than 14 days after 50 percent of the control seedlings have emerged</i></p> <p><i>Vegetative Vigor: Five plants per replicate are recommended.</i></p>
Number of seeds/plants/species/replicate	<p>Seedling Emergence: Four replicates for each species. Wheat onion and turnip had 2 pots per replicate with 5 seeds per pot. Sorghum had 5 pots per replicate with 2 seeds per pot.</p> <p>Vegetative Vigor: Four replicate for each species. Cucumber and sorghum had 5 pots per replicate with 2 plants per pot; turnip had 2 pots per replicates with 5 plants per pot.</p>	<p><i>Seedling Emergence: Ten seeds per replicate should be used.</i></p> <p><i>OECD recommends a minimum of five seeds planted in each replicate within 24 hours of incorporation of the test substance. All seeds of each species for each test should be of the same size class. The seed should not be imbibed.</i></p> <p><i>Vegetative Vigor: Four replicates per dose should be used.</i></p> <p><i>OECD recommends a minimum of four replicates per treatment.</i></p>
Number of plants retained after thinning	Vegetative Vigor: Cucumber sorghum 3 seeds were planted per pot and thinned to 2 plants prior to treatment. Turnip 7 seeds were planted per pot and thinned to 5 plants prior to treatment.	
<u>Number of replicates</u> Control: Adjuvant control: Treated:	4 4 4	<p><i>Four replicates per dose should be used.</i></p> <p><i>OECD recommends a minimum of four replicates per treatment</i></p>

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Parameters	Seedling Emergence/Vegetative Vigor	
	Details	Remarks
		Criteria
<u>Test concentrations (lb ai/A)</u>  Nominal:         Measured:	0 (negative), 0 (solvent), 0.004, 0.008, 0.016, 0.032, 0.064, 0.128 and 0.256 lb ai/A Flufenacet + Metribuzin; reviewer-calculated nominal Flufenacet concentrations of 0 (negative), 0 (solvent), 0.0032, 0.0064, 0.013, 0.026, 0.051, 0.102 and 0.21 lb ai/A Flufenacet.  Measured Axiom 68 DF/A was not reported.  Reviewer-calculated measured concentrations of Flufenacet were 0 (negative), 0 (solvent), 0.0030, 0.0059, 0.012, 0.024, 0.048, 0.097 and 0.19 lb ai/A Flufenacet.	Nominal calculated Metribuzin concentrations were 0 (negative), 0 (solvent), 0.00082, 0.0016, 0.0033, 0.0065, 0.013, 0.026 and 0.052 lb ai/A Metribuzin.  Measured Metribuzin concentrations were 0 (negative), 0 (solvent), 0.00075, 0.0015, 0.0032, 0.0060, 0.012, 0.024 and 0.048 lb ai/A Metribuzin.  <i>Five test concentrations should be used with a dose range of 2X or 3X progression</i>  <i>OECD recommends three concentrations, preferably with application rates equivalent to 0.0 (control), 1.0, 10.0 and 100 mg substance per kg of oven-dried soil.</i>
<u>Method and interval of analytical verification</u>  LOQ:  LOD:	Analysis via HPLC with C18, RP column with UV detection (250 nm for Flufenacet and 313 nm for Metribuzin). Flufenacet: 0.93 mg/L Metribuzin: 0.24 mg/L Not reported.	
Adjuvant (type, percentage, if used)	Formulation Blank	
<u>Test container (pot)</u>  Size/Volume Material: (glass/polystyrene)	Pots with diameter of 9 cm for wheat, turnip and onion and 10.5 cm diameter for sorghum and cucumber. Plastic	<i>Non-porous containers should be used.</i>  <i>OECD recommends that non-porous plastic or glazed pot be used.</i>
Growth facility	Greenhouse	
Method/depth of seeding	Method of planting seeds, not reported; Soil top watered initially, then bottom watered	

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Parameters	Seedling Emergence/Vegetative Vigor	
	Details	Remarks
		Criteria
	thereafter.	
<u>Test material application</u> Application time including the plant growth stage  Number of applications  Application interval  Method of application	Seedling Emergence: after planting. Vegetative Vigor: at the 2-4 leaf stage  1  N/A- single application  The test material was applied using an Allen spray chamber equipped with an overhead TeeJet 80015 EVH nozzle, applied 13 inches above the soil surface.	
<u>Details of soil used</u> Geographic location Depth of soil collection Soil texture % sand % silt % clay pH: % organic carbon CEC Moisture at 1/3 atm (%)	N/A N/A Sandy loam 60 24 16 5.7 Not reported Not reported Not reported	Soil was steam pasteurized.  Organic matter: 2.5%  <hr/> <i>Soil mixes containing sandy loam, loam, or clay loam soil with no greater than 2% organic matter are preferable. Glass beads, rock wool, and 100% acid washed sand are not preferred.</i>  <i>OECD prefers the soil to be sieved (0.5 cm) to remove coarse fragments. Carbon content should not exceed 1.5% (3% organic matter). Fine particles (under 20um) makeup should be between 10 and 20%. The recommended pH is between 5.0 and 7.5.</i>
Details of nutrient medium, if used	N/A	
<u>Watering regime and schedules</u>	Deionized water to prepare dose solution and city water thereafter (Johnson County, Kansas)	

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Parameters	Seedling Emergence/Vegetative Vigor	
	Details	Remarks
		Criteria
Water source/type: Volume applied: Interval of application: Method of application:	Not reported. Daily. The plants were bottom watered daily.	<i>EPA prefers that bottom watering be utilized for seedling emergence studies so that the chemical is not leached out of the soil during the test.</i>
Any pest control method/fertilization, if used	N/A	
<u>Test conditions</u> Temperature: Photoperiod: Light intensity and quality:  Relative humidity:	Day: 18-29°C 15L:9D Natural sunlight supplemented with artificial light. 300 watts/m <sup>2</sup> 24-98%	Actual ranges of specific test conditions were not reported.  <i>EPA prefers that the cold vs warm loving plants be tested in two separate groups to optimize plant growth.</i>  <i>OECD prefers that the temperature, humidity and light conditions be suitable for maintaining normal growth of each species for the test period.</i>
<u>Reference chemical (if used)</u> Name: Concentrations:	N/A	
Other parameters, if any	None	

**2. Observations:**

**Table 4: Observation Parameters - Seedling Emergence/Vegetative Vigor.**

Parameters	Seedling Emergence/Vegetative Vigor	
	Details	Remarks
Parameters measured (e.g., number of germinated seeds, emerged seedlings, plant height, dry weight or other endpoints)	- Emergence (Seedling Emergence only) - Survival - Dry weight - Phytotoxicity	
Measurement technique for each parameter	Emergence (Seedling emergence only) and phytotoxicity were visually determined.	

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	Survival was defined as the percent of emerged by the study author. Seedlings were cut at soil level. Total plant weight was estimated measuring the total dry weight per replicate following oven drying. Height was measured by extending cut seedlings to maximum length.	
Observation intervals	Each pot was inspected weekly, emergence and phytotoxicity assessments performed. Dry weight and height was recorded at study termination.	
Other observations, if any	N/A	
Were raw data included?	Yes	
Phytotoxicity rating system, if used	0=No injury or effect; 20%=Slight plant effect or more effect restricted to one area (i.e. one leaf); 40%=Moderate effects involving the whole plant; 60%=Severe effect with recovery possible; 80%=Total plant effect, very poor vigor; 100%=Moribund or plant death.	

**II. RESULTS and DISCUSSION:**

**A. INHIBITORY EFFECTS:** Study author results presented in Tables 5 and 6 are in terms of nominal Flufenacet lb ai/A, which were calculated from nominal concentrations of Flufenacet + Metribuzin lb ai/A. The study author did not quantify inhibitions, and the treatment levels showing significant effects are based on the NOAEC. The reviewer's results reported in Table 7 and 9 are in terms of measured lb ai/A Flufenacet, which were obtained from measured Metribuzin calculations back-calculated to determine Flufenacet concentrations (Flufenacet measurements on spray solutions were not valid due to analytical problems). The reviewer's results reported in Table 8 and 10 are based on measured concentrations of Metribuzin.

**Seedling Emergence:**

The study author reported results are based on the pooled (negative and solvent) controls. Pooled control emergence ranged from 74 to 100%. The study author and reviewer reported no significant inhibitions in emergence for any species tested ( $p>0.05$ ).

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The study author reported % survived based on number emerged. The study author reported pooled control survival based on number emerged ranged from 97 to 100%. There were inhibitions in survival for all species except wheat. There were significant inhibitions for sorghum at the 0.064 lb ai Axiom 68 DF/A (0.051 lb ai/A Flufenacet); for turnip at the 0.032, 0.064 and 0.128 lb ai Axiom 68 DF/A (0.026, 0.051 and 0.103 lb ai/A Flufenacet); and for onion at the 0.064 and 0.128 lb ai Axiom 68 DF/A (0.051 and 0.103 lb ai/A Flufenacet); inhibitions were not quantified (Dunnett's test,  $p < 0.05$ ).

The reviewer measured seedling survival based on number planted. Negative control survival based on number planted ranged from 78 to 100%. The reviewer observed significant inhibitions in survival at the same treatment levels that the study author found. Significant inhibitions in turnip measured 32, 76 and 92% at the 0.024, 0.048 and 0.097 lb ai/A Flufenacet treatment levels, respectively, compared to the negative control and exhibited a dose-response (Williams test,  $p < 0.05$ ). Significant inhibitions in onion survival were 37 and 63% at the 0.048 and 0.097 lb ai/A Flufenacet treatment levels, respectively; sorghum significant inhibitions were observed at the highest treatment level only, 0.048 lb ai/A Flufenacet, and measured 48% (Williams test,  $p < 0.050$ ).

The study author and reviewer found significant inhibitions in seedling height in all species. There were significant inhibitions in onion height at the 0.032, 0.064 and 0.128 lb ai/A Axiom 68 DF/A (0.026, 0.051 and 0.103 lb ai/A Flufenacet) (Dunnett's test,  $p < 0.05$ ). There was also significant inhibitions compared to the pooled control for sorghum height at the 0.032 and 0.064 lb ai/A Axiom 68 DF/A (0.026 and 0.051 lb ai/A Flufenacet) (Dunnett's test,  $p < 0.05$ ). Significant inhibitions for turnip and wheat height were found at the highest concentration only, 0.128 lb ai Axiom 68 DF/A (0.103 lb ai/A Flufenacet) (Dunnett's test,  $p < 0.05$ ). The reviewer found significant inhibitions in onion height starting at a lower treatment level, and were 10, 20, 35 and 40% at the 0.012, 0.024, 0.048 and 0.097 lb ai/A Flufenacet treatment levels, respectively. The reviewer noted significant inhibitions in sorghum height also started at a lower treatment level and measured 12, 22 and 37% at the 0.012, 0.024 and 0.048 lb ai/A Flufenacet treatment levels, respectively (Williams test,  $p < 0.05$ ). Significant inhibitions in turnip and wheat were observed at the highest treatment level only, 0.097 lb ai/A Flufenacet, and measured 50 and 10%, respectively, compared to the negative control (Williams test,  $p < 0.05$ ).

The study author found inhibitions in dry weight in all species except wheat. Significant inhibitions in both onion and turnip weight were observed at the 0.032, 0.064 and 0.128 lb ai/A Axiom 68 DF/A (0.026, 0.051 and 0.103 lb ai/A Flufenacet) (Dunnett's test,  $p < 0.05$ ). Significant inhibitions compared to the pooled control for sorghum weight were found at the highest concentration only, 0.064 lb ai/A Axiom 68 DF/A (0.051 lb ai/A Flufenacet) (Dunnett's test,  $p < 0.05$ ).

The reviewer found significant inhibitions for dry weight in all species compared to the negative control. There were significant inhibitions in onion of 34, 62 and 75% at the 0.024, 0.048 and 0.096 lb ai Flufenacet/A; in sorghum of 27, 44 and 69% at the 0.012, 0.024 and 0.048 lb ai/A Flufenacet; in turnip of 25, 77 and 94% at the 0.024, 0.048 and 0.096 lb ai/A Flufenacet; and in wheat of 14% at the 0.097 lb ai/A Flufenacet compared to the negative control (Williams test,  $p < 0.05$ ). There was also a significant inhibition in the solvent control compared to the negative control for sorghum (30%; t-test,  $p = 0.0237$ ).

Based on the study author's seedling emergence results, the most sensitive monocot species was onion based on dry weight, with NOAEC and IC<sub>25</sub> values of 0.016 and 0.024 lb ai/A Axiom 68 DF/A (0.013 and 0.019 lb ai/A Flufenacet, respectively); and the most sensitive dicot species was turnip based on dry weight, with NOAEC and IC<sub>25</sub> values of 0.016 and 0.025 lb ai/A Axiom 68 DF/A (0.013 and 0.020 lb ai/A Flufenacet, respectively).

The occurrence of phytotoxic effects increased in severity and prevalence with increasing test

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concentrations for all species except wheat. Maximum effects were 75, 60 and 100% for onion, sorghum and turnip, respectively. Effects were dose-related.

### Vegetative Vigor

The reviewer could not validate the seedling survival, height and dry weight data for cucumber and turnip in this study because it appears that either dead plants were measured, or the survival data was in error. As a result, only the sorghum data was assessed. The study author and reviewer both found no significant inhibitions in seedling survival for sorghum compared to the pooled or negative control.

The study author found significant inhibitions compared to the pooled control for sorghum height at the 0.064 and 0.128 lb ai Axiom 68 DF/A (0.051 and 0.103 lb ai/A Flufenacet). The reviewer observed significant effects in seedling height at the same treatment levels. Significant decreases in seedling height in sorghum were 11 and 34% at the 0.048 and 0.097 lb ai/A Flufenacet treatment levels, respectively (Williams test,  $p < 0.05$ ).

The study author did not find any significant effects on sorghum dry weight. However, the reviewer found significant inhibitions in seedling dry weight in sorghum compared to the negative control of 15, 23, 21 and 32% at the 0.012, 0.024, 0.048 and 0.097 lb ai/A Flufenacet/A treatment levels, respectively (Williams test,  $p < 0.05$ ). There was also a significant difference between the negative control and the solvent control for sorghum dry weight (19%; t-test,  $p = 0.0298$ ).

Based on the study author's sorghum results, the most sensitive endpoint for sorghum was based on height, with NOAEC and IC<sub>25</sub> values of 0.026 and 0.083 lb ai/A Flufenacet (0.032 and 0.104 lb ai/A Axiom 68 DF/A, respectively). The most sensitive dicot species could not be determined due to invalid data.

The occurrence of phytotoxic effects increased in severity and prevalence with increasing test concentrations. Maximum effects were 40% for sorghum. Effects were dose-related.

### B. REPORTED STATISTICS:

Emergence (seedling emergence only), survival, dry weight, and height means and standard deviations were determined for control and treatment groups. Treatments were compared to the pooled control. NOEC and LOEC, and EC<sub>25</sub> and EC<sub>50</sub> determined using SAS software (SAS, version 6.12, 1996). Control means were compared using two-tailed comparison t-test and two-tailed Fisher's Exact test (emergence and survival data only). If pooling the control groups was unacceptable, then blank formulation control was used. Normality of homogeneity of variance was determined using Shapiro-Wilk and Leven's test, respectively (99% certainty). If assumptions were violated, then data were arcsine transformed (phytotoxicity and emergence/survival) or log transformed (height and dry weight). Control means were compared to treatment means using Dunnett's test. If assumptions of homogeneity and normality were violated ( $p \leq 0.01$ ) with transformed values, then comparison used nonparametric Dunnett's test on rank of values within each block. ECx values were determined using probit model (emergence/survival) or four-parameter logistic or cumulative normal models (height, dry weight and phytotoxicity ratings). EC<sub>50</sub> concentrations were also estimated using trimmed Spearman-Kärber statistic. The model with the tightest confidence intervals were reported. Nominal concentrations for the formulation Axiom 68 DF were used for all analyses. Note: the study author results presented in Tables 5 and 6 are in terms of nominal Flufenacet lb ai/A, which were calculated from nominal concentrations of Flufenacet + Metribuzin lb ai/A. The study author did not quantify inhibitions, and the treatment levels showing significant effects are based on the NOEC.



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**Table 5: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for height (lb ai/A Flufenacet)									
	height (cm)	NOEC	IC <sub>05</sub>	95%CI	IC <sub>25</sub>	95%CI	IC <sub>50</sub>	95%CI	slope	95%CI
Onion <sup>1</sup>	7.33-12.1	0.013	ND	N/A	0.037	0.022-0.063	>0.102	N/A	N/A	N/A
Sorghum <sup>2</sup>	19.4-32.5	0.013	ND	N/A	0.030	0.022-0.042	>0.051	N/A	N/A	N/A
Turnip <sup>3</sup>	7.25-14.7	0.051	ND	N/A	0.075	0.061-0.091	>0.102	>0.102	N/A	N/A
Wheat <sup>3</sup>	29.4-32.6	0.051	ND	N/A	>0.102	N/A	>0.102	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

<sup>1</sup> Significant inhibitions in onion height at the 0.032, 0.064 and 0.128 lb ai Axiom 68 DF/A (0.026, 0.051 and 0.103 lb ai/A Flufenacet) (Dunnett's test, p<0.05).

<sup>2</sup> Significant inhibitions compared to the pooled control for sorghum height at the 0.032 and 0.064 lb ai Axiom 68 DF/A (0.026 and 0.051 lb ai/A Flufenacet) (Dunnett's test, p<0.05).

<sup>3</sup> Significant inhibitions compared to the pooled control for turnip and wheat height at the 0.128 lb ai Axiom 68 DF/A (0.103 lb ai/A Flufenacet) (Dunnett's test, p<0.05).

**Table 5a: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for biomass (lb ai/A Flufenacet)									
	weight (mg)	NOEC	IC <sub>05</sub>	95%CI	IC <sub>25</sub>	95%CI	IC <sub>50</sub>	95%CI	slope	95%CI
Onion <sup>1</sup>	0.0195-0.0775	0.013	ND	N/A	0.019	0.0088-0.041	0.042	0.026-0.067	N/A	N/A
Sorghum <sup>2</sup>	0.249-0.872	0.026	ND	N/A	0.0255	0.012-0.053	0.042	0.028-0.063	N/A	N/A
Turnip <sup>3</sup>	0.099-1.83	0.013	ND	N/A	0.020	0.015-0.027	0.032	0.026-0.038	N/A	N/A
Wheat	0.724-0.859	0.102	ND	N/A	>0.102	N/A	>0.102	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

<sup>1</sup> Significant inhibitions in onion weight at the 0.032, 0.064 and 0.128 lb ai Axiom 68 DF/A (0.026, 0.051 and 0.103 lb ai/A Flufenacet) (Dunnett's test, p<0.05).

<sup>2</sup> Significant inhibitions compared to the pooled control for sorghum weight at the 0.064 lb ai Axiom 68 DF/A (0.051 lb ai/A Flufenacet) (Dunnett's test, p<0.05).

<sup>3</sup> Significant inhibitions compared to the pooled control for turnip weight at the 0.032, 0.064 and 0.128 lb ai Axiom 68 DF/A (0.026, 0.051 and 0.103 lb ai/A Flufenacet) (Dunnett's test, p<0.05).

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**Table 5b: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for emergence (lb ai/A Flufenacet)									
	%	NOEC	EC <sub>05</sub>	95% CI	EC <sub>25</sub>	95% CI	EC <sub>50</sub>	95% CI	slope	95% CI
Onion	83-98	0.102	ND	N/A	>0.102	N/A	>0.102	N/A	N/A	N/A
Sorghum	63-75	0.051	ND	N/A	>0.051	N/A	>0.051	N/A	N/A	N/A
Turnip	94-100	0.102	ND	N/A	>0.102	N/A	>0.102	N/A	N/A	N/A
Wheat	90-100	0.102	ND	N/A	>0.102	N/A	>0.102	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

**Table 5c: Effect of Axiom 68 DF (54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for survival (lb ai/A Flufenacet); based on # emerged									
	%	NOEC	EC <sub>05</sub>	95% CI	EC <sub>25</sub>	95% CI	EC <sub>50</sub>	95% CI	slope	95% CI
Onion <sup>1</sup>	36-100	0.026	ND	N/A	0.044	0.033-0.054	0.072	0.059-0.093	ND	ND
Sorghum <sup>2</sup>	72-100	0.026	ND	N/A	0.043	0.031-0.082	>0.051	N/A*	ND	ND
Turnip <sup>3</sup>	8-100	0.013	ND	N/A	0.025	0.020-0.029	0.037	0.031-0.042	ND	ND
Wheat	95-100	0.102	ND	N/A	>0.102	N/A	>0.102	N/A	ND	ND

ND- Not determined. NC- Not calculable.

\*The confidence interval was wider than the range of concentrations tested.

<sup>1</sup> Significant inhibitions in onion survival at the 0.064 and 0.128 lb ai Axiom 68 DF/A (0.051 and 0.103 lb ai/A Flufenacet) (Dunnett's test, p<0.05).

<sup>2</sup> Significant inhibitions in sorghum survival at the 0.064 lb ai Axiom 68 DF/A (0.051 lb ai/A Flufenacet) (Dunnett's test, p<0.05).

<sup>3</sup> Significant inhibitions in turnip survival at the 0.032, 0.064 and 0.128 lb ai Axiom 68 DF/A (0.026, 0.051 and 0.103 lb ai/A Flufenacet) (Dunnett's test, p<0.05).

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**Table 6: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Vegetative Vigor**

Species	Results summary for height (lb ai/A Flufenacet)									
	height (cm)	NOEC	IC <sub>05</sub>	95%CI	IC <sub>25</sub>	95%CI	IC <sub>50</sub>	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum	49.9-75.6	0.026	ND	N/A	0.083	0.075-0.091	>0.102	N/A	N/A	N/A
Turnip	ND	0.032	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

<sup>1</sup> Significant inhibitions compared to the pooled control for sorghum height at the 0.064 and 0.128 lb ai Axiom 68 DF/A (0.051 and 0.103 lb ai/A Flufenacet).

**Table 6a: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Vegetative Vigor**

Species	Results summary for biomass (lb ai/A Flufenacet) <sup>1</sup>									
	weight (mg)	NOEC	IC <sub>05</sub>	95%CI	IC <sub>25</sub>	95%CI	IC <sub>50</sub>	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum	17-25.1	0.102	NC	N/A	>0.102	N/A	>0.102	N/A	N/A	N/A
Turnip	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

\*NOEC could not be determined; therefore estimated by Probit method (EC<sub>05</sub>).

**Table 6b: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Vegetative Vigor**

Species	Results summary for survival (lb ai/A Flufenacet)									
	%	NOEC	EC <sub>05</sub>	95%CI	EC <sub>25</sub>	95%CI	EC <sub>50</sub>	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum	100	0.102	NC	N/A	>0.102	N/A	>0.102	N/A	N/A	N/A
Turnip	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

\*The confidence interval was wider than the range of concentrations tested.

**Data Evaluation Record on the Acute Toxicity of Formulated Flufenacet (Axiom 68 DF, 54.4% a.i. Flufenacet, 13.8% a.i. Metribuzin) to Terrestrial Vascular Plants: Seedling Emergence and Vegetative Vigor**

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Mid-study emergence					
Seedling Emergence					
Control	Onion	Sorghum	Turnip	Wheat	Formulation Blank
80-100	83-98	63-80	93-100	90-100	68-100

Plant Injury Index*										
Seedling Emergence						Vegetative Vigor				
Control	Onion	Sorghum	Turnip	Wheat	Formulation Blank	Control	Cucumber	Sorghum	Turnip	Formulation Blank
0	0-75	0-60	0-100	0-5	0	0	0-100	0-40	0-100	0

0=No injury or effect; 20%=Slight plant effect or more effect restricted to one area (i.e. one leaf); 40%=Moderate effects involving the whole plant; 60%=Severe effect with recovery possible; 80%=Total plant effect, very poor vigor; 100%=Moribund or plant death.

**C. VERIFICATION OF STATISTICAL RESULTS BY THE REVIEWER:**

All analyses were conducted comparing treated to the negative control. These analyses were conducted using CETIS version 1.8.7.12 and backend settings approved for use by EFED on 3/25/14. Data for each endpoint were tested to determine if their distributions were normal and if their variances were homogeneous using Shapiro-Wilk's and Levene's tests, respectively. Data that satisfied these assumptions were subjected to Dunnett's and William's tests, and data that did not satisfy these assumptions were subjected to the non-parametric Mann-Whitney U and Jonckheere's tests. Significant differences between the negative and solvent controls were determined by Equal Variance Two-Sample t-test. Reviewer calculated measured concentrations for Flufenacet (based on Metribuzin concentrations) were used for all analyses. Linear (survival and emergence) and nonlinear (height and dry weight) regression models were used to interpret EC/ICx values. Negative control was compared to solvent control using equal variance t-test ( $p < 0.05$ ). Note: the reviewer's results reported in Table 7 and 9 are in terms of measured lb ai/A Flufenacet, which were obtained from measured Metribuzin calculations back-calculated to determine Flufenacet concentrations (Flufenacet measurements on spray solutions were not valid due to analytical problems). The reviewer's results reported in Table 8 and 10 are based on measured concentrations of Metribuzin.

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**Table 7: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for height (lb ai/A Flufenacet)									
	height (cm)	NOEC	IC <sub>05</sub>	95% CI	IC <sub>25</sub>	95% CI	IC <sub>50</sub>	95% CI	slope	95% CI
Onion <sup>1</sup>	7.33-12.1	0.0059	0.00354	0.000519-0.00748	0.0331	0.0255-0.0423	0.157	0.103-0.239	N/A	N/A
Sorghum <sup>2</sup>	19.4-32.5	0.0059	0.00575	0.00234-0.00894	0.0256	0.0211-0.0307	0.0724	0.0527-0.0995	N/A	N/A
Turnip <sup>3</sup>	7.25-14.7	0.048	0.0442	N/A-0.057	0.0712	0.0584-0.083	0.0991	0.0873-0.112	N/A	N/A
Wheat <sup>4</sup>	29.4-32.6	0.048	0.03	0.00898-0.064	0.856*	N/A-11.2*	NC	N/A	N/A	N/A

ND- Not determined. NC- Not calculable

\*Endpoints and/or confidence intervals are outside the tested range of concentrations and should be interpreted cautiously.

<sup>1</sup> Significant decrease in onion height, inhibitions of 10, 20, 35 and 40% at the 0.012, 0.024, 0.048 and 0.097 lb ai/A treatment, respectively, compared to the negative control (Williams test, p<0.05).

<sup>2</sup> Significant decrease in sorghum height, inhibitions of 12, 22 and 37% at the 0.012, 0.024 and 0.048 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05).

<sup>3</sup> Significant decrease in turnip height, inhibition of 50% at the 0.097 lb ai/A treatment compared to the negative control (Williams test, p<0.05).

<sup>4</sup> Significant decrease in wheat height, inhibitions of 10% at the 0.097 lb ai/A treatment compared to the negative control (Williams test, p<0.05).

**Table 7a: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for biomass (lb ai/A Flufenacet)									
	weight (g)	NOEC	IC <sub>05</sub>	95% CI	IC <sub>25</sub>	95% CI	IC <sub>50</sub>	95% CI	slope	95% CI
Onion <sup>1</sup>	0.00523-0.00879	0.024	0.00184	N/A-0.0101	0.032	0.0169-0.0559	0.233	0.0621-0.873	N/A	N/A
Sorghum <sup>2</sup>	0.062-0.116	0.0059	0.00337	N/A-0.00768	0.0175	0.0114-0.0251	0.0549	0.035-0.0862	N/A	N/A
Turnip <sup>3</sup>	0.0633-0.19	0.048	0.0484	N/A-0.0651	0.068	N/A-0.0879	0.0861	0.074-0.1	N/A	N/A
Wheat	0.782-0.088	0.097	NC	N/A	>0.097	N/A	>0.097	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

<sup>1</sup> Significant decrease in onion weight, inhibition of 41 and 31% at the 0.048 and 0.097 lb ai/A treatments, respectively, compared to the negative control (Dunnett's test, p<0.05).

<sup>2</sup> Significant decrease in sorghum weight, inhibitions of 18, 31 and 40% at the 0.012, 0.024 and 0.048 lb ai/A treatments, respectively, compared to negative control (Williams test, p<0.05).

<sup>3</sup> Significant decrease in turnip weight, inhibitions of 61% at the 0.097 lb ai/A treatment compared to the negative control (Dunnett's test, p<0.05).

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**Table 7b: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for emergence (lb ai/A Flufenacet)									
	%	NOEC	EC <sub>05</sub>	95%CI	EC <sub>25</sub>	95%CI	EC <sub>50</sub>	95%CI	slope	95%CI
Onion	83-98	0.097	NC	N/A	>0.097	N/A	>0.097	N/A	N/A	N/A
Sorghum	63-80	0.048	NC	N/A	>0.048	N/A	>0.048	N/A	N/A	N/A
Turnip	93-100	0.097	>0.097	N/A	>0.097	N/A	>0.097	N/A	N/A	N/A
Wheat	90-100	0.097	NC	N/A	>0.097	N/A	>0.097	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

**Table 7c: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for survival (lb ai/A Flufenacet); based on # planted									
	%	NOEC	EC <sub>05</sub>	95%CI	EC <sub>25</sub>	95%CI	EC <sub>50</sub>	95%CI	slope	95%CI
Onion <sup>1</sup>	33-93	0.024	0.00573	0.00251-0.00916	0.0218	0.0153-0.0287	0.0552	0.0414-0.0824	1.67	1.16-2.19
Sorghum <sup>2</sup>	40-78	0.024	0.000136	N/A-0.000854	0.00384*	0.000366-0.00772	0.0391	0.0198-0.356	0.669	0.242-1.1
Turnip <sup>3</sup>	8-100	0.012	0.0127	0.00904-0.0159	0.0225	0.0184-0.0264	0.0335	0.0288-0.0391	3.9	2.98-4.83
Wheat	90-100	0.097	NC	N/A	>0.097	N/A	>0.097	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

\*EC<sub>25</sub> is less than the NOAEC.

<sup>1</sup> Significant decrease in onion survival, inhibition of 37 and 63% at the 0.048 and 0.097 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05).

<sup>2</sup> Significant decrease in sorghum survival, inhibition of 48% at the 0.048 lb ai/A treatment compared to the negative control (Williams test, p<0.05). The difference between the solvent control compared to the negative control was 24% and was just below the action level for significant difference of 25%.

<sup>3</sup> Significant decrease in turnip survival, inhibition of 32, 76 and 92% at the 0.024, 0.048 and 0.097 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05).

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**Table 8: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for height (lb ai/A Metribuzin)									
	height (cm)	NOEC	IC <sub>05</sub>	95%CI	IC <sub>25</sub>	95%CI	IC <sub>50</sub>	95%CI	slope	95%CI
Onion <sup>1</sup>	7.33-12.1	0.0015	0.00090	0.00013-0.00190	0.00840	0.00647-0.011	0.040	0.026-0.061	N/A	N/A
Sorghum <sup>2</sup>	19.4-32.5	0.0015	0.00146	0.00059-0.0023	0.00649	0.0054-0.0078	0.018	0.013-0.025	N/A	N/A
Turnip <sup>3</sup>	7.25-14.7	0.012	0.011	N/A-0.014	0.0181	0.015-0.021	0.025	0.022-0.028	N/A	N/A
Wheat <sup>4</sup>	29.4-32.6	0.012	0.0076	0.0023-0.016	0.217*	N/A-2.84*	NC	N/A	N/A	N/A

ND- Not determined. NC- Not calculable

\*Endpoints and/or confidence intervals are outside the tested range of concentrations and should be interpreted cautiously.

<sup>1</sup> Significant decrease in onion height, inhibitions of 10, 20, 35 and 40% at the 0.0032, 0.0060, 0.012 and 0.024 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05).

<sup>2</sup> Significant decrease in sorghum height, inhibitions of 12, 22 and 37% at the 0.0032, 0.0060 and 0.012 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05).

<sup>3</sup> Significant decrease in turnip height, inhibition of 50% at the 0.024 lb ai/A treatment compared to the negative control (Williams test, p<0.05).

<sup>4</sup> Significant decrease in wheat height, inhibitions of 10% at the 0.024 lb ai/A treatment compared to the negative control (Williams test, p<0.05).

**Table 8a: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for biomass (lb ai/A Metribuzin)									
	weight (g)	NOEC	IC <sub>05</sub>	95%CI	IC <sub>25</sub>	95%CI	IC <sub>50</sub>	95%CI	slope	95%CI
Onion <sup>1</sup>	0.00523-0.00879	0.0060	0.000467	N/A-0.0026	0.0081	0.0043-0.014	0.059	0.016-0.221	N/A	N/A
Sorghum <sup>2</sup>	0.062-0.116	0.0015	0.00085	N/A-0.0019	0.00444	0.0029-0.0064	0.014	0.0089-0.022	N/A	N/A
Turnip <sup>3</sup>	0.0633-0.19	0.012	0.0123	N/A-0.017	0.0172	N/A-0.0223	0.218	0.019-0.025	N/A	N/A
Wheat	0.782-0.088	0.024	NC	N/A	>0.024	N/A	>0.024	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

<sup>1</sup> Significant decrease in onion weight, inhibition of 41 and 31% at the 0.012 and 0.024 lb ai/A treatments, respectively, compared to the negative control (Dunnett's test, p<0.05).

<sup>2</sup> Significant decrease in sorghum weight, inhibitions of 18, 31 and 40% at the 0.0032, 0.0060 and 0.012 lb ai/A treatments, respectively, compared to negative control (Williams test, p<0.05).

<sup>3</sup> Significant decrease in turnip weight, inhibitions of 61% at the 0.024 lb ai/A treatment compared to the negative control (Dunnett's test, p<0.05).

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**Table 8b: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for emergence (lb ai/A Metribuzin)									
	%	NOEC	EC <sub>05</sub>	95%CI	EC <sub>25</sub>	95%CI	EC <sub>50</sub>	95%CI	slope	95%CI
Onion	83-98	0.024	NC	N/A	>0.024	N/A	>0.024	N/A	N/A	N/A
Sorghum	63-80	0.012	NC	N/A	>0.012	N/A	>0.012	N/A	N/A	N/A
Turnip	93-100	0.024	>0.024	N/A	>0.024	N/A	>0.024	N/A	N/A	N/A
Wheat	90-100	0.024	NC	N/A	>0.024	N/A	>0.024	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

**Table 8c: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Seedling Emergence**

Species	Results summary for survival (lb ai/A Metribuzin); based on # planted									
	%	NOEC	EC <sub>05</sub>	95%CI	EC <sub>25</sub>	95%CI	EC <sub>50</sub>	95%CI	slope	95%CI
Onion <sup>1</sup>	33-93	0.0060	0.0015	0.00064-0.0023	0.00553	0.0039-0.0073	0.014	0.011-0.021	0.424	0.294-0.556
Sorghum <sup>2</sup>	40-78	0.0060	0.000034	N/A-0.00022	0.000974*	0.000093-0.00196	0.0099	0.0050-0.0903	0.170	0.061-0.279
Turnip <sup>3</sup>	8-100	0.0030	0.0032	0.0023-0.0040	0.0057	0.0047-0.0067	0.0085	0.0073-0.010	0.989	0.756-1.225
Wheat	90-100	0.024	NC	N/A	>0.024	N/A	>0.024	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

\*EC<sub>25</sub> is less than the NOAEC.

<sup>1</sup> Significant decrease in onion survival, inhibition of 37 and 63% at the 0.012 and 0.024 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05).

<sup>2</sup> Significant decrease in sorghum survival, inhibition of 48% at the 0.012 lb ai/A treatment compared to the negative control (Williams test, p<0.05). The difference between the solvent control compared to the negative control was 24% and was just below the action level for significant difference of 25%.

<sup>3</sup> Significant decrease in turnip survival, inhibition of 32, 76 and 92% at the 0.0060, 0.012 and 0.024 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05).



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**Table 9: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Vegetative Vigor**

Species	Results summary for height (lb ai/A Flufenacet)									
	height (cm)	NOEC	IC <sub>05</sub>	95%CI	IC <sub>25</sub>	95%CI	IC <sub>50</sub>	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum <sup>1</sup>	49.9-75.6	0.024	0.0327	0.0231-0.0403	0.0775	0.0713-0.0839	0.141	0.121-0.165	N/A	N/A
Turnip	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

<sup>1</sup> Significant decrease in sorghum height, inhibitions of 11 and 34% at the 0.048 and 0.097 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05).

**Table 9a: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Vegetative Vigor**

Species	Results summary for biomass (lb ai/A Flufenacet)									
	weight (g)	NOEC	IC <sub>05</sub>	95%CI	IC <sub>25</sub>	95%CI	IC <sub>50</sub>	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum <sup>1</sup>	1.7-2.51	0.0059	0.000958	N/A-0.00698	0.0491	0.0253-0.089	0.757*	0.081-7.07*	N/A	N/A
Turnip	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

<sup>1</sup> Significant decrease in sorghum weight, inhibitions of 15, 23, 21 and 32% at the 0.012, 0.024, 0.048 and 0.097 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05); also significant difference in the solvent control compared to the negative control (19%) (t-test, p=0.0298).

**Table 9b: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Vegetative Vigor**

Species	Results summary for survival (lb ai/A Flufenacet); based on # planted									
	%	NOEC	EC <sub>05</sub>	95%CI	EC <sub>25</sub>	95%CI	EC <sub>50</sub>	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum	100	0.097	>0.097	N/A	>0.097	N/A	>0.097	N/A	N/A	N/A
Turnip	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

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**Table 10: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Vegetative Vigor**

Species	Results summary for height (lb ai/A Metribuzin)									
	height (cm)	NOEC	IC <sub>05</sub>	95%CI	IC <sub>25</sub>	95%CI	IC <sub>50</sub>	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum <sup>1</sup>	49.9-75.6	0.0060	0.0083	0.0059-0.010	0.0197	0.018-0.021	0.036	0.031-0.042	N/A	N/A
Turnip	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

<sup>1</sup> Significant decrease in sorghum height, inhibitions of 11 and 34% at the 0.012 and 0.024 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05).

**Table 10a: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Vegetative Vigor**

Species	Results summary for biomass (lb ai/A Metribuzin)									
	weight (g)	NOEC	IC <sub>05</sub>	95%CI	IC <sub>25</sub>	95%CI	IC <sub>50</sub>	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum <sup>1</sup>	1.7-2.51	0.0015	0.00024	N/A-0.0018	0.012	0.0064-0.023	0.192*	0.021-1.79*	N/A	N/A
Turnip	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

\*Endpoints and/or confidence intervals are outside the tested range of concentrations and should be interpreted cautiously.

<sup>1</sup> Significant decrease in sorghum weight, inhibitions of 15, 23, 21 and 32% at the 0.0032, 0.0060, 0.012 and 0.024 lb ai/A treatments, respectively, compared to the negative control (Williams test, p<0.05); also significant difference in the solvent control compared to the negative control (19%) (t-test, p=0.0298).

**Table 10b: Effect of Axiom 68 DF (a.i. 54.4% Flufenacet + 13.8% Metribuzin) on 21-Day Vegetative Vigor**

Species	Results summary for survival (lb ai/A Metribuzin); based on # planted									
	%	NOEC	EC <sub>05</sub>	95%CI	EC <sub>25</sub>	95%CI	EC <sub>50</sub>	95%CI	slope	95%CI
Cucumber	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A
Sorghum	100	0.024	>0.024	N/A	>0.024	N/A	>0.024	N/A	N/A	N/A
Turnip	ND	ND	ND	N/A	ND	N/A	ND	N/A	N/A	N/A

ND- Not determined. NC- Not calculable.

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Mid-study emergence					
Seedling Emergence					
Control	Onion	Sorghum	Turnip	Wheat	Formulation Blank
80-100	83-98	63-80	95-100	90-100	68-100

Plant Injury Index*										
Seedling Emergence						Vegetative Vigor				
Control	Onion	Sorghum	Turnip	Wheat	Formulation Blank	Control	Cucumber	Sorghum	Turnip	Formulation Blank
0	0-75	0-60	0-100	0-5	0	0	0-100	0-40	0-100	0

0=No injury or effect; 20%=Slight plant effect or more effect restricted to one area (i.e. one leaf); 40%=Moderate effects involving the whole plant; 60%=Severe effect with recovery possible; 80%=Total plant effect, very poor vigor; 100%=Moribund or plant death.

**Seedling Emergence: Flufenacet**

**Monocot**

**Most sensitive monocot: Sorghum, based on dry weight**

EC <sub>50</sub> /IC <sub>50</sub> : 0.0549 lb ai/A Flufenacet	95% C.I.: 0.035-0.0862 lb ai/A Flufenacet
EC <sub>25</sub> /IC <sub>25</sub> : 0.0175 lb ai/A Flufenacet	95% C.I.: 0.0114-0.0251 lb ai/A Flufenacet
EC <sub>05</sub> /IC <sub>05</sub> : 0.00337 lb ai/A Flufenacet	95% C.I.: N/A-0.00768 lb ai/A Flufenacet
NOEC: 0.0059 lb ai/A Flufenacet	
Slope: N/A	95% C.I.: N/A

**Dicot**

**Most sensitive dicot: Turnip, based on survival**

EC <sub>50</sub> /IC <sub>50</sub> : 0.0335 lb ai/A Flufenacet	95% C.I.: 0.0288-0.0391 lb ai/A Flufenacet
EC <sub>25</sub> /IC <sub>25</sub> : 0.0225 lb ai/A Flufenacet	95% C.I.: 0.0184-0.0264 lb ai/A Flufenacet
EC <sub>05</sub> /IC <sub>05</sub> : 0.0127 lb ai/A Flufenacet	95% C.I.: 0.00904-0.0159 lb ai/A Flufenacet
NOEC: 0.012 lb ai/A Flufenacet	
Slope: 3.9	95% C.I.: 2.98-4.83

**Seedling Emergence: Metribuzin**

**Monocot**

**Most sensitive monocot: Sorghum, based on dry weight**

EC <sub>50</sub> /IC <sub>50</sub> : 0.014 lb ai/A Metribuzin	95% C.I.: 0.0089-0.022 lb ai/A Metribuzin
EC <sub>25</sub> /IC <sub>25</sub> : 0.00444 lb ai/A Metribuzin	95% C.I.: 0.0029-0.0064 lb ai/A Metribuzin

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EC <sub>05</sub> /IC <sub>05</sub> : 0.00085 lb ai/A Metribuzin	95% C.I.: N/A-0.0019 lb ai/A Metribuzin
NOEC: 0.0015 lb ai/A Metribuzin	
Slope: N/A	95% C.I.: N/A

**Dicot**

**Most sensitive dicot: Turnip, based on survival**

EC <sub>50</sub> /IC <sub>50</sub> : 0.0085 lb ai/A Metribuzin	95% C.I.: 0.0073-0.010 lb ai/A Metribuzin
EC <sub>25</sub> /IC <sub>25</sub> : 0.0057 lb ai/A Metribuzin	95% C.I.: 0.0047-0.0067 lb ai/A Metribuzin
EC <sub>05</sub> /IC <sub>05</sub> : 0.0032 lb ai/A Metribuzin	95% C.I.: 0.0023-0.0040 lb ai/A Metribuzin
NOEC: 0.0030 lb ai/A Metribuzin	
Slope: 0.989	95% C.I.: 0.756-1.225

**Vegetative Vigor: Flufenacet**

**Monocot**

**Most sensitive monocot: Sorghum, based on dry weight**

EC <sub>50</sub> /IC <sub>50</sub> : 0.757 lb ai Flufenacet/A	95% C.I.: 0.081-7.07 lb ai Flufenacet/A
EC <sub>25</sub> /IC <sub>25</sub> : 0.0491 lb ai Flufenacet/A	95% C.I.: 0.0253-0.089 lb ai Flufenacet/A
EC <sub>05</sub> /IC <sub>05</sub> : 0.000958 lb ai Flufenacet/A	95% C.I.: N/A-0.0156 lb ai Flufenacet/A
NOEC: 0.0059 lb ai Flufenacet/A	
Slope: N/A	95% C.I.: N/A

**Dicot**

**Most sensitive dicot: Could not be determined, invalid data for dicots**

EC <sub>50</sub> /IC <sub>50</sub> : N/A	95% C.I.: N/A
EC <sub>25</sub> /IC <sub>25</sub> : N/A	95% C.I.: N/A
EC <sub>05</sub> /IC <sub>05</sub> : N/A	95% C.I.: N/A
NOEC: N/A	
Slope: N/A	95% C.I.: N/A

**Vegetative Vigor: Metribuzin**

**Monocot**

**Most sensitive monocot: Sorghum, based on dry weight**

EC <sub>50</sub> /IC <sub>50</sub> : 0.192 lb ai/A Metribuzin	95% C.I.: 0.021-1.793 lb ai/A Metribuzin
EC <sub>25</sub> /IC <sub>25</sub> : 0.0125 lb ai/A Metribuzin	95% C.I.: 0.0064-0.023 lb ai/A Metribuzin
EC <sub>05</sub> /IC <sub>05</sub> : 0.00024 lb ai/A Metribuzin	95% C.I.: N/A-0.0018 lb ai/A Metribuzin
NOEC: 0.0015 lb ai/A Metribuzin	
Slope: N/A	95% C.I.: N/A

**Dicot**

**Most sensitive dicot: Could not be determined, invalid data for dicots**

EC <sub>50</sub> /IC <sub>50</sub> : N/A	95% C.I.: N/A
EC <sub>25</sub> /IC <sub>25</sub> : N/A	95% C.I.: N/A
EC <sub>05</sub> /IC <sub>05</sub> : N/A	95% C.I.: N/A
NOEC: N/A	
Slope: N/A	95% C.I.: N/A

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**D. STUDY DEFICIENCIES:**

2. Applying to both the Seedling Emergence and Vegetative Vigor study, analytical confirmation of Flufenacet test concentrations was invalid. Measured Flufenacet recoveries were low (44-68%) as were the method validation recoveries (29-39%). The study author determined the analysis was invalid due to a filtration step that trapped Flufenacet particles, introducing error into the analytical measurement. Therefore, to obtain the measured Flufenacet application rates, the reviewer back calculated Flufenacet concentrations from the measured Metribuzin concentrations (Metribuzin recoveries were valid, 91-97%; method validation recoveries 90-94%).
3. Applying to both the Seedling Emergence and Vegetative Vigor study, the study author reported that sorghum, wheat, onion and cucumber had previously been identified to be the most sensitive of the ten crops tested with Flufenacet (Johns, 1994). Turnip, onion and cucumber had previously been identified to be the most sensitive of 10 crops tested with Metribuzin (Bürge, 1992). This study utilized 3 monocots and 1 dicot for the seedling emergence study and 1 monocot and 2 dicots for the vegetative vigor study. EPA recommends four monocots in two families, including corn and six dicots in four families, including soybean and a root crop.
4. Applying to both the Seedling Emergence and Vegetative Vigor study, the study author presented data for seedling dry weight as a total weight and did not take into account number of seedlings per replicate.
5. Mean control seedling survival was 78% for sorghum in the Seedling Emergence study, which is lower than the OCSPP recommended mean seedling survival of 90%. Historic germination rates were not provided.
6. In the Seedling Emergence study, sorghum EC<sub>25</sub> for survival was much lower than the NOAEC and may be related to the solvent (24% different between the negative control survival and solvent control survival) and/or low seedling emergence and survival. The reviewer selected sorghum dry weight as the endpoint instead of sorghum survival because sorghum dry weight had a definitive dose-response.
7. In the Vegetative Vigor Study, height and weight measurements were provided for treatment level data with up to 100% mortality, which means dead plants may have been measured and weighed and the data included in the report or there was an error the survival data. There was no way to correct the data.
8. The physico-chemical properties of the test material were not reported. Soil % organic carbon, CEC and moisture content were not reported.

**E. REVIEWER'S COMMENTS:**

The reviewer and study author's most sensitive species were not in agreement for the seedling emergence test. The study author's most sensitive monocot species was onion based on dry weight, with NOAEC and IC<sub>25</sub> values of 0.013 and 0.019 lb ai/A Flufenacet, respectively (0.016 and 0.024 lb ai/A Axiom 68 DF); the study author's most sensitive dicot species was turnip based on dry weight, with NOAEC and IC<sub>25</sub> values of 0.013 and 0.020 lb ai/A Flufenacet, respectively (0.016 and 0.025 lb ai/A Axiom 68 DF). The reviewer's most sensitive monocot was sorghum based on dry weight, with NOAEC and IC<sub>25</sub> values of 0.0059 and 0.0175 lb ai Flufenacet/A, respectively; and the most sensitive dicot species was turnip based on survival, with NOAEC and EC<sub>25</sub> values of 0.012 and 0.0225 lb ai/A Flufenacet, respectively.

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For the vegetative vigor test, the study author's most sensitive endpoint for sorghum was based on height, with NOAEC and IC<sub>25</sub> values of 0.026 and 0.083 lb ai/A Flufenacet, respectively (0.032 and 0.104 lb ai/A Axiom 68 DF). The reviewer's most sensitive endpoint for sorghum was based on dry weight, with NOAEC and IC<sub>25</sub> values of 0.0059 and 0.0491 lb ai/A Flufenacet, respectively. The most sensitive dicot species could not be determined due to lack of valid data for survival, height and weight.

The study author determined results using pooled controls and application rates based on the Axiom 68 DF formulation containing both Metribuzin and Flufenacet, while the reviewer determined results based on the negative control and application rates for Flufenacet only. The study author also calculated effects on total dry weight, not taking survival into account. These differences likely resulted in the differences in the most sensitive species.

Also note, the reviewer found sorghum survival had a lower IC<sub>25</sub>/EC<sub>25</sub> than sorghum dry weight, however sorghum survival EC<sub>25</sub> was less than the NOAEC and may have been influenced by the solvent and low seedling emergence and survival. Sorghum dry weight had a definitive dose-response, and the reviewer selected sorghum dry weight as the endpoint. The reviewer's results are presented in the Executive Summary and Conclusions sections of this DER.

The in-life portion of this study was conducted from November 23 to December 14, 1999.

### **F. CONCLUSIONS:**

#### **Seedling Emergence**

For seedling emergence, the most sensitive monocot species was sorghum based on dry weight, with NOAEC and IC<sub>25</sub> values of 0.0059 and 0.0175 lb ai/A Flufenacet, respectively; and the most sensitive dicot species was turnip based on survival, with NOAEC and EC<sub>25</sub> values of 0.012 and 0.0225 lb ai/A Flufenacet, respectively. In terms of the other active ingredient, Metribuzin, NOAEC and IC<sub>25</sub> values for sorghum dry weight were 0.0015 and 0.00444 lb ai/A Metribuzin, respectively, and the NOAEC and EC<sub>25</sub> values for turnip survival were 0.0030 and 0.0057 lb ai/A Metribuzin, respectively.

Based on reviewer calculated total formulation concentrations, the NOAEC and IC<sub>25</sub> values for sorghum based on dry weight were 0.011 and 0.0322 lb/A total formulation, respectively, and the NOAEC and EC<sub>25</sub> values for turnip based on survival were 0.022 and 0.041 lb/A total formulation, respectively.

#### **Vegetation Vigor**

For vegetative vigor, the most sensitive monocot species was sorghum based on dry weight, with NOAEC and IC<sub>25</sub> values of 0.0059 and 0.0491 lb ai/A Flufenacet, respectively. The most sensitive dicot species could not be determined due to lack of valid data for height and weight. In terms of the other active ingredient, Metribuzin, NOAEC and IC<sub>25</sub> values for sorghum dry weight were 0.0015 and 0.0125 lb ai/A Metribuzin, respectively.

Based on reviewer calculated total formulation concentrations, the NOAEC and IC<sub>25</sub> values for sorghum based on dry weight were 0.011 and 0.090 lb/A total formulation, respectively. The most sensitive dicot species could not be determined due to lack of valid data for height and weight.

#### **Seedling emergence - Flufenacet**

Most sensitive monocot and IC<sub>25</sub>: Sorghum (dry weight, 0.0322 lb ai/A Flufenacet).

Most sensitive dicot and EC<sub>25</sub>: Turnip (survival, 0.0225 lb ai/A Flufenacet).

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**Seedling emergence - Metribuzin**

Most sensitive monocot and IC<sub>25</sub>: Sorghum (dry weight, 0.00444 lb ai/A Metribuzin).

Most sensitive dicot and EC<sub>25</sub>: Turnip (survival, 0.0057 lb ai/A Metribuzin).

**Seedling emergence – Total Formulation**

Most sensitive monocot and IC<sub>25</sub>: Sorghum (dry weight, 0.0322 lb/A Total Formulation).

Most sensitive dicot and EC<sub>25</sub>: Turnip (survival, 0.041 lb/A Total Formulation).

**Vegetative Vigor – Flufenacet**

Most sensitive monocot and IC<sub>25</sub>: Sorghum (dry weight, 0.0491 lb ai/A Flufenacet).

Most sensitive dicot and EC<sub>25</sub>: Could not be determined due to invalid data.

**Vegetative Vigor – Metribuzin**

Most sensitive monocot and IC<sub>25</sub>: Sorghum (dry weight, 0.0125 lb ai/A Metribuzin).

Most sensitive dicot and EC<sub>25</sub>: Could not be determined due to invalid data.

**Vegetative Vigor – Total Formulation**

Most sensitive monocot and IC<sub>25</sub>: Sorghum (dry weight, 0.090 lb/A Total Formulation).

Most sensitive dicot and EC<sub>25</sub>: Could not be determined due to invalid data.

**III. REFERENCES:**

1. Burge, C. L. 1992. Tier 2 Seed Germination, Seed Emergence and Seedling Vigor Nontarget Phytotoxicity Study Using Metribuzin. Bayer Report No. 103800. MRID 42447803.
2. Gyllings Software, Pesticide Research Manager Version 5. Gylling Data Management Inc., Brookings, South Dakota.
3. Johns, C. L. 1994. Tier 2 Seed Germination, Seedling Emergence and Vegetative Vigor Nontarget Phytotoxicity Study Using FOE 5043. Bayer Report No. 106780. MRID 43465002.
4. Kratkg, B. A. and Warren, G. E., 1971, The Use of Three Simple, Rapid, Bioassays on Forty Two Herbicides, Weed Research, 11, 257-262.
5. Microsoft Excel 97 SR-1. 1997. Microsoft Corporation.
6. SAS Institute. 1996. SAS/BASE/STAT, Version 6.12 Cary, North Carolina.
7. USEPA, 1982. Pesticide Assessment Guidelines, Subdivision J - Hazard Evaluation: Nontarget Plants. EPA-540/9-82--020. Office of Pesticide Programs, Washington, D. C. 55 pp.
8. USEPA, 1986. Standard Evaluation Procedure, Non-Target Plants: Seed Germination, Seedling Emergence and Vegetative Vigor - Tier 1. EPA-540/9-86-134. Office of Pesticides Programs, Washington, D.C.
9. USEPA, 1989. Pesticide Programs; Good Laboratory Practice Standards; Final Rule (40 CFR Part 160). Federal Register, Vol. 54, No. 158: 34067 - 34074.